An Oath/Declaration in compliance with 37 C.F.R. § 1.67(a) will be submitted upon receipt of the executed Oath/Declaration.

The Examiner rejected claims 25-26 under 35 U.S.C. § 112, second paragraph. The amendments to claim 25 render this rejection moot. Therefore, withdrawal of the § 112(2) rejection is respectfully requested.

The Examiner also rejected claims 1, 5-6 and 25-26 under 35 U.S.C. § 102(b) as being anticipated by Castrucci et al. (J. Virol., 69, 2725 (1995)). The Examiner further rejected claims 1, 3-4, 6, and 25-26 under 35 U.S.C. § 102(b) as being anticipated by Sweet et al. (J. Virol. Methods, 69, 103 (1997)). In addition, the Examiner rejected claims 1-2, 5-6, 9, and 25 under 35 U.S.C. § 102(b) as being anticipated by Park et al. (J. Virol., 72, 2449 (1998)). These rejections, as they may be maintained with respect to the pending claims, are respectfully traversed.

Castrucci et al. disclose the preparation of four transfectant influenza viruses. The transfectant viruses were generally obtained by infecting MDBK cells with influenza A/equine/Miami/63 (Eq/MIA), an amantadine-sensitive strain, then transfecting those cells with a complex of M RNA obtained by transcription of a M cDNA derived from influenza A/Puerto Rico/8/34 (PR8), an amantadine-resistant strain, and nucleoprotein (NP) and polymerase proteins from influenza A/duck/Alberta/35/76, with subsequent culturing in MDCK cells in the presence of amantadine (page 2726). Each of the four viruses was prepared with a different M RNA; one virus was prepared with full length PR8 M RNA (control), another with RNA having a deletion corresponding to the 5 carboxy-terminal residues of PR8 M ("COOH-5"), yet another with RNA having a deletion corresponding to the 10 carboxy-terminal residues of PR8 M ("COOH-10"), and yet another with RNA corresponding to a deletion of the last residue of PR8 M ("COOH-1") (page 2726).

Castrucci et al. report that virus was only rescued from cells transfected with a complex having PR8 M RNA or COOH-1 RNA (page 2726), and that the COOH-1 virus was rescued with the same efficiency as that of the control virus (Eq/MIA-PR8M). Castrucci et al. also disclose that COOH-1 virus had a ten-fold lower titer and replicated for a shorter time than the control virus in infected ferrets, and retained sufficient immunogenicity to protect animals against challenge with the control virus (page 2726).

Castrucci et al. do not disclose a recombinant influenza virus comprising an ion channel protein comprising a mutation in the transmembrane domain, a host cell comprising such a virus, or a method of preparing such a virus via only recombinant DNA.

Sweet et al. disclose that amantadine-resistant viruses were prepared by two different methods. One method employed plaquing an amantadine-sensitive virus on MDCK cells in the presence of amantadine (page 104). This method resulted in the recovery of at least 17 clones, 7 of which had a mutation at a residue corresponding to residue 30 in M2 and 10 of which had a mutation at a residue corresponding to residue 31 in M2 (Table 2).

The other method employed infecting MDBK cells with helper virus A/AA/6/60 E10 and, 1 hour later, transfecting those cells with viral ribonucleoprotein and M RNA obtained by transcription of a M cDNA from a cold adapted A/AA/6/60 (amantadine-sensitive) virus which had been mutagenized at three codons (codons for amino acid residues 27, 30, and 31) hypothesized to be responsible for amantadine resistance in several type A strains (abstract and page 104). Transfected material was plaqued on MDCK cells in the presence of amantadine (page 107). Twenty clones were recovered, but only 3/20 had virus with the anticipated sequence alterations (pages 107 and 108). It is further disclosed that only one of the clones, 18B, was a pure population (Table 2). Notably, the titer of 18B was three logs lower than the parent virus even when grown in the absence of amantadine (Table 1 and page 110). Sweet et al. further disclose that the new virus clones will be tested in animal models to determine the significance of the cold adapted M gene in the attenuation phenotype of the A/AA/6/60 vaccine (page 109).

Sweet et al. do not teach a recombinant influenza virus comprising an ion channel comprising a mutation in the transmembrane domain, which mutation does not alter the *in vitro* replication of the virus but is associated with attenuation of the virus *in vivo*, a host cell comprising the virus, or a method of preparing such a virus solely with recombinant DNA.

To determine which regions of M2 are responsible for its incorporation into virions, Park et al. transfected COS cells with COOH-10 M2 RNA (see Castrucci et al.) or M2 RNA which lacked the region corresponding to the entire cytoplasmic tail, and then infected those cells with Ty/MN influenza virus. It is disclosed that the M2 protein mutant which lacked the entire cytoplasmic tail was poorly expressed at the cell surface and poorly incorporated into virions

(page 2452). In contrast, COOH-10 M2 was detected at the cell surface and in virions (page 2452). Based on the results in Park et al., and in view of the reported failure to rescue COOH-10 virus in Castrucci et al., the authors of Park et al. conclude that the defect in COOH-10 is at a step in virus replication other than incorporation into virions.

Park et al. also prepared influenza virus with a chimeric protein comprising M2 and Sendai virus F protein. Six chimeras were prepared, in which the extracellular, transmembrane or cytoplasmic domain of M2 was replaced with the corresponding region of F protein. To prepare virus, chimeric M2 RNA was transfected into cells and those cells infected with Ty/MN virus. It is disclosed that only three of the M2 chimeras were expressed on the cell surface, and that only one of the chimeras was incorporated into virions ("MFF", having the extracellular domain of M2 and the transmembrane and cytoplasmic domains of the F protein). Virus titers for the MFF chimera were not disclosed. Using an antibody specific for PR8 M2 and another specific for the cytoplasmic tail of M2, Park et al. showed that the MFF chimera does not form hetero-oligomers with Ty/MN M2.

Park et al. do not teach a recombinant influenza virus comprising an ion channel protein comprising a mutation in the transmembrane region, which mutation does not alter the *in vitro* replication of the virus, or a method of preparing such a virus which employs only recombinant DNA.

Accordingly, withdrawal of the § 102 rejections is respectfully requested.

Conclusion

Applicant respectfully submits that the claims are in condition for allowance and notification to that effect is earnestly requested. The Examiner is invited to telephone Applicant's attorney (612-373-6959) to facilitate prosecution of this application.

If necessary, please charge any additional fees or credit overpayment to Deposit Account No. 19-0743.

Respectfully submitted,

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Date February 4, 2003 By _____
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Reg. No. 39,665

CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail, in an envelope addressed to: Commissioner of Patents, Washington, D.C. 20231, on this 4th day of February, 2003.

Dawn M. Poole
Name

Dawn M. Poole
Signature